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US-PAT-NO: 6146842
DOCUMENT-IDENTIFIER: US 6146842 A

TITLE: High-throughput screening assays utilizing metal-chelate capture

DATE-ISSUED: November 14, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/15; 435/4, 530/200, 530/300, 530/329

CLAIMS:

What is claimed is:
1. An assay method for determining the effect of a substance on the activity of an enzyme, comprising the steps of:
a. combining a test compound with an enzyme capable of catalyzing the transfer of a detectable moiety to a substrate, a donor substance labeled with the detectable label, and a substrate specific for the enzyme comprising a metal-chelating moiety, and incubating the mixture under conditions sufficient to promote the enzymatic reaction;
b. contacting the combination formed in (a) with a metal-derivatized solid phase under conditions sufficient to promote reaction of the immobilized metal and the chelating moiety; and
c. detecting the signal generated.
2. The method of claim 1 wherein the enzyme comprises a prenyl transferase.
3. The method of claim 2 wherein the enzyme comprises farnesyl transferase or geranylgeranyl transferase.
4. The method of claim 2 wherein donor substance comprises a substance comprising a prenyl group capable of being transferred to a substrate by the action of the prenyl transferase.
5. The method of claim 2 wherein the substrate comprises a peptide or protein capable of being modified by the action of the prenyl transferase.
6. The method of claim 1 wherein the metal chelating moiety is a metal chelating peptide.
7. The method of claim 6 wherein the peptide comprises six histidine residues.
8. The method of claim 1 wherein the metal is selected from the group consisting of nickel, calcium, palladium, platinum, ruthenium and rhodium.
9. The method of claim 1 wherein the detectable label comprises a radioactive label.
10. The method of claim 9 wherein the radioactive label is selected from the group consisting of .sup.3 H, .sup.14 C, .sup.35 S, .sup.125 I, .sup.45 Ca, .sup.33 P and .sup.32 P.
11. The method of claim 1 wherein the metal derivatized substrate comprises a solid phase having a scintillant disposed thereon and a metal immobilized thereon.

12. An assay method for determining the effect of a substance on the activity of a prenyl transferase enzyme, comprising the steps of:
a. combining a test compound with a prenyl transferase enzyme, a radioactively-labeled donor substance, and a substrate specific for the prenyl transferase enzyme comprising a metal-chelating moiety, and incubating the mixture under conditions sufficient to promote the enzymatic reaction;

b. contacting the combination formed in (a) with a metal-derivatized solid phase under conditions sufficient to promote reaction of the immobilized metal and the chelating moiety; and
c. detecting the signal generated.
13. The method of claim 12 wherein the enzyme comprises farnesyl transferase or geranylgeranyl transferase.
14. The method of claim 12 wherein donor substance comprises a substance comprising a prenyl group capable of being transferred to a substrate by the action of the prenyl transferase.
15. The method of claim 12 wherein the substrate comprises a peptide or protein capable of being modified by the action of the prenyl transferase.
16. The method of claim 12 wherein the metal chelating moiety comprises six histidine residues.
17. The method of claim 12 wherein the metal is nickel.
18. The method of claim 12 the radioactive label is selected from the group consisting of .sup.3 H, .sup.14 C, .sup.35 S, 125I, .sup.45 Ca, .sup.33 P and .sup.32 P.
19. The method of claim 12 wherein the metal derivatized substrate comprises a solid phase having a scintillant disposed thereon and a metal immobilized thereon.

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BSPR:
The Ras family of proteins are important in the signal transduction pathway modulating cell growth. The protein is produced in the ribosome, released into the cytosol, and post-translationally modified. The first step in the series of post-translational modifications is the alkylation of Cys.sup.168 with farnesyl or geranylgeranyl pyrophosphate in a reaction catalyzed by prenyl transferase enzymes such as farnesyl transferase and geranylgeranyl transferase (Hancock, J. F., et al., Cell, 57:1167-1177 (1989)). Subsequently, the three C-terminal amino acids are cleaved (Gutierrez, L., et al., EMBO J. 8:1093-1098 (1989)), and the terminal Cys is converted to a methyl ester (Clark, S., et al., Proc. Nat'l Acad Sci. (USA), 85:4643-4647 (1988)). Some forms of Ras are also reversibly palmitoylated on cysteine residues immediately N-terminal to Cys.sup.168 (Buss, J. E., et al., Mol. Cell. Biol., 6:116-122 (1986)). It is believed that these modifications increase the hydrophobicity of the C-terminal region of Ras, causing it to localize at the surface of the cell membrane. Localization of Ras to the cell membrane is necessary for signal transduction (Willumsen, B. M., et al., Science, 310:583-586 (1984)).

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USPT	l24 and (surface or solid or plate or bead or immobilize or support)	8	L26
USPT	l24 and l22	0	L25
USPT	l23 and (covalent$ or co-valent$)	8	L24
USPT	(posttranslation$ or post-translation$).ti.	8	L23
USPT	l21 and enzym$	5	L22
USPT	l19 or l20	21	L21
USPT	l16 and solid.clm.	6	L20
USPT	l16 and surface.clm.	16	L19
USPT	l16 and bead.clm.	1	L18
USPT	l16 and immobil$.clm.	0	L17
USPT	(posttranslation$ or post-translation$ or translation$).clm. and (modulat$ or modif$).ti.	142	L16
USPT	l14 and immobil$	7	L15
USPT	l13 same (covalent$ or co-valent$)	12	L14
USPT	l11 same (polypeptides or poly-peptides)	294	L13
USPT	l11 same (polypeptide or poly-peptide)	294	L12
USPT	l5 same(antagon$ or block$ or inhibit$ or modif$ or candid$ or competit$)	1678	L11
USPT	l9 and (antagon$ or block$ or inhibit$ or modif$ or candid$ or competit$)	67	L10
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USPT	l6 and (covalent$ or co-valent$)	647	L7
USPT	l5 and (polypeptide or poly-peptide)	1300	L6
USPT	(posttranslation$ or post-translation$ or translation$)same (modulat$ or modif$)same enzym$	1721	L5
USPT	('5846765')[PN]	1	L4
USPT	l1 and covalent$.clm.	9	L3
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USPT	(posttranslation$ or post-translation$ or translation$).clm. and (modulat$ or modif$).clm.	1479	L1